# Effect of Oceanographic Environments on Sexual Maturation, Salinity Tolerance, and Vasotocin Gene Expression in Homing Chum Salmon

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# Effect of Oceanographic Environments on Sexual Maturation, Salinity Tolerance, and Vasotocin Gene Expression in Homing Chum Salmon

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**ABSTRACT**—Effects of the coastal oceanographic conditions on sexual maturation, salinity tolerance, and expression of vasotocin (VT) gene in homing chum salmon, *Oncorhynchus keta*, were studied by four years of fieldwork and transfer experiments. In fieldwork in 1992 and 1993, fish were sampled at three sites along their migratory pathway on the Sanriku coast, Japan. In transfer experiments in 1994 and 1995, fish captured in the seawater (SW) environment were transferred to SW or freshwater (FW) aquaria and sampled 1 to 4 days later. The distribution of cold and warm currents, which governs the oceanographic conditions of the Sanriku coast, were deduced from the mean sea surface temperature. Maturity of homing fish was estimated by gonadal states. Salinity tolerance was estimated by plasma $Na^+$ levels and mortality in SW environment. Changes in VT gene expression were assessed by a quantitative dot blot analysis of the hypothalamic levels of VT mRNA. Homing fish were fully mature, and showed high plasma $Na^+$ levels and high mortality in the SW environment in 1992 and 1994 when the warm current dominated. In the 1994 transfer experiment, VT mRNA levels markedly increased in the SW-retained males, whereas the levels were decreased by FW-transfer in both sexes. Homing fish were not fully matured in 1993 and 1995 when the branch of cold current reached the Sanriku coast. In the 1995 transfer experiment, VT mRNA levels did not change in both SW-retained and FW-transferred fish. In conclusion, the warmer oceanographic conditions affected the maturity and salinity tolerance in homing salmon, which in turn altered the osmotically-induced expression pattern of VT gene.

## INTRODUCTION

A teleost neurohypophysial hormone, vasotocin (VT), is synthesized by neurosecretory cells in the preoptic nucleus, and is involved in osmoregulation and reproductive behavior (Urano *et al*., 1994). Anadromous salmonids undergo freshwater (FW) adaptation and gonadal maturation, when they return to their natal river. Roles of VT in salmonid spawning migration are thus of particular interests.

In terms of teleostean osmoregulation, various lines of evidence indicated that VT may function in FW adaptation (Urano *et al*., 1994). The plasma levels of VT at the peak of diurnal variation in FW-adapted fish were significantly higher than those in brackish water-adapted fish at the same period (Kulczykowska and Stolarski, 1996). Nonetheless, an osmoregulatory role of VT in sexually mature fish has remained unclear. In chum salmon, coho salmon, and brook trout, gonadal maturation accompanied a loss of salinity tolerance, that is, increased plasma $Na^+$ levels and high mortality were observed when homing fish were retained in seawater (SW) (Sower and Schreck, 1982; McCormick and Naiman, 1985; Hirano *et al*., 1990). Such alteration in osmoregulatory capacity during final maturation may modulate the VT system in pre-spawning chum salmon.

On the Sanriku coast of the Pacific Ocean, Japan, homing chum salmon complete gonadal maturation just before entering the river, because their spawning ground is about 1 km upstream from the river mouth. Thus, a loss of salinity tolerance can occur before the fish enter the river if the returning fish is fully mature. A retrospective survey of our previous observation on the Sanriku population of chum salmon actually showed annual variations of maturity, salinity tolerance, and expression of VT gene. Interestingly, distribution of cold and warm currents in the Sanriku coast showed corresponding annual variation, suggesting that annual varia-

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tion of regional oceanographic conditions changed expression of VT gene. Accordingly, year on year comparison of results obtained from wild fish will provide indispensable information to clarify the effects of sexual maturation and salinity tolerance on the VT system.

In the present study, we examined VT gene expression in pre-spawning chum salmon obtained by two years of fieldwork and two years of transfer experiments in which maturity and salinity tolerance of returning fish varied with the annual variation of regional oceanographic conditions. Since each experiment was performed independently, detailed experimental procedure was not necessarily identical to one another. Nonetheless, such comparison showed that regional oceanographic conditions had possible effects on maturity and salinity tolerance, which in turn modulated the expression of VT gene. In addition, we determined plasma levels of 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) as a possible endocrine factor which regulate the expression of VT gene in fully mature fish. A part of the present results was published previously (Hiraoka *et al.*, 1996).

## MATERIALS AND METHODS

### Regional oceanography

Oceanographic condition in the Sanriku coast is governed mainly by relative strengths of the Kurile Current and the Japan Current, major cold and warm currents in the north-west Pacific Ocean near Japan. Annual variation in the relative distribution of the currents was inferred by comparing the distribution of mean sea surface temperature on the Sanriku coast in late November from 1992 to 1995. To illustrate the annual variation, pseudo color images were reconstituted on the contour map of mean sea surface temperature obtained from "*Quick Bulletin of Ocean Conditions*" which is published periodically by Hydrographic Department, Maritime Safety Agency, Japan.

### Fieldwork in 1992 and 1993

*Animals.* From late November to early December in 1992 and 1993, pre-spawning chum salmon, *Oncorhynchus keta*, of both sexes were captured at three sites along their migratory pathway on the Sanriku coast of the Pacific Ocean, in the northern part of Honshu island, Japan. The fish were captured by a salmon set-net placed 1 km outside the Otsuchi bay (Ocean fish), a net set in the bay close to the mouth of the Otsuchi river (Bay fish), and a trap set in the Otsuchi river 500 m upstream from the river mouth (River fish). Generally, the Ocean fish have not fully developed a characteristic nuptial color of mature fish and some fish still show a silver color, whereas the Bay and River fish have completely developed the nuptial color. In the Bay and River, most of the female fish have already ovulated and the males contain much more milt than the Ocean fish. The River fish were sampled in the field near the trap. The Ocean and Bay fish were transported by oxygenated-SW tank (90 cm×140 cm×95 cm) to the Otsuchi Marine Research Center, Ocean Research Institute, University of Tokyo. The fish were transferred to aerated-running SW aquaria (290 cm×150 cm×100 cm, 12°C, 100 liter/min) and sampled on the following day.

*Sample collection.* Fish were anesthetized with 0.02% tricaine methane sulfonate (MS-222, Sigma) and the fork length, body weight, and gonad weight were measured. Blood was taken from the caudal vessel, left on ice for more than 1 hr, and centrifuged at 3,000 rpm for 15 min to separate the plasma. Immediately after blood sampling, the fish were decapitated and the brain tissue containing the hypothalamus was taken out, frozen in liquid nitrogen and stored at –80°C. Entire magnocellular neurosecretory nuclei were included in this tissue block. Plasma $Na^+$ levels were determined with an electrolyte analyzer (AVL984-S, Graz, Austria). The maturity of homing fish was estimated by gonadosomatic index (GSI, gonad weight/body weight ×100).

*Northern hybridization.* Total RNA was extracted from the tissue sample by the acid guanidinium thiocyanate-phenol chloroform method (Chomczynski and Sacchi, 1987). The 1/20 volume of total RNA (ca. 10 μg) from individual fish was electrophoresed in a 1% agarose/formaldehyde gel, and transferred to a Hybond-$N^+$ membrane (Amersham International plc, Amersham, Bucks, U.K.) according to the manufacturer's instruction. A cDNA probe was prepared by a random priming method using a Multiprime DNA labeling system and [α-$^{32}$P]dCTP (Amersham) with chum salmon VT-I cDNA as a template. Hybridization with the labeled probe was performed in a 6 × standard saline citrate (SSC), 0.1% sodium dodecyl sulfate (SDS), 5 × Denhardt's reagent and 100 μg/ml denatured yeast tRNA at 60°C for 16 hr. The membranes were washed twice with 0.1× SSC/0.1% SDS at 60°C for 1 hr, and exposed to a Fuji imaging plate (Fuji Photo Film, Tokyo, Japan) for 3 hr. Hybridization signals were analyzed by a Bioimaging analyzer (Fuji Photo Film). The radioactivity of the signals was recorded as the intensity of photostimulated luminescence (PSL) after subtraction of background. All samples were analyzed in a single assay within each year. The values shown in the present report were not normalized, because the results did not change from those obtained by the use of data normalized with β-actin mRNA levels (data not shown).

*Enzyme immuno assay.* The plasma levels of 17α,20β-dihydroxy-4-pregnen-3-one (DHP) in the fish of 1993 fieldwork were determined by an enzyme immunoassay, basically following the method of Asahina *et al.* (1995). Plasma samples were extracted with diethyl ether, and the extracts were evaporated to dryness with nitrogen gas. The dry residues were reconstituted with assay buffer (0.05 M borate buffer, pH 7.8, containing 0.1% BSA and 0.01% thimerosal). The standards and samples were incubated with anti-DHP antiserum and HRP-labeled DHP (FKA 332 and FKA 331, Cosmo Bio Co. Ltd, Japan) at 4°C for 16 hr in a microtiter plate coated with goat anti-rabbit IgG (ICN Pharmaceuticals Inc, Aurora, Ohio). After washing with 0.9% NaCl, a substrate solution (0.5 mg/ml *o*-phenylenediamine, 0.01% $H_2O_2$ in 0.2 M citrate buffer pH 4.5) was added and incubated at room temperature (RT) for 30 min. The reaction was stopped by addition of 0.6 N $H_2SO_4$ and the absorbance at 492 nm was measured with a microplate reader (MTP-120, Corona Electric Co. Ltd, Japan). The sensitivity of the assay was 1 ng/ml, and the intra- and inter-assay coefficients of variances (C.V.) were 9.6% and 7.6%, respectively.

### Transfer experiments in 1994 and 1995

*Animals.* In early December in 1994 and 1995, pre-spawning chum salmon of both sexes were captured by the salmon set-net near the mouth of the Otsuchi bay (the same site referred to as Ocean in the fieldwork) and transported to the aquaria as described in the fieldwork. They were maintained for a day in a calm condition to recover from handling stresses of the capture and transportation. Then the initial control fish (day 0) were sampled. Afterward, they were divided into two groups, those retained in SW and others whose environmental running SW was replaced with running FW (11°C, 100 liter/min). The levels of $Cl^-$ in the aquaria, monitored with a chloridometer (Buchler), showed that SW was almost completely replaced by FW within 1 hr. The SW and FW fish were sampled 1, 2, and 4 days (1994) or 1 and 3 days (1995) after the experimental treatments.

To confirm that fish recovered from the handling stresses, the plasma concentrations of cortisol and $Cl^-$ in control fish were determined by an enzyme immuno assay and an electrolyte analyzer, respectively. The plasma cortisol levels were 411.7±87.6 in males and 692.3±43.6 in females in 1994, whereas the levels were

229.6±44.9 in males and 482.5±115.0 in females in 1995 (means ± sem; ng/ml). The plasma $Cl^-$ levels were 148.0±4.5 in males and 153.7±7.0 in females in 1994, whereas the levels were 151.8±3.5 in males and 151.2 ±2.5 in females in 1995 (means ± sem; mmol/l). The plasma cortisol and $Cl^-$ concentrations were within the range observed in the field fish of previous research (Hirano *et al*., 1990), indicating that the present fish did not suffer or almost completely recovered from the handling stresses. Sample collection and total RNA extraction were performed as described in the fieldwork. The plasma $Na^+$ levels and DHP levels were also determined as in the fieldwork. For the assay of DHP levels, the intra- and inter-assay C.V. were 7.4% and 10.6%, respectively.

*Quantitative hybridization analyses.* In the 1994 experiment, the levels of VT mRNA were quantitatively analyzed by Northern blot hybridization as described previously (Hiraoka *et al*., 1997). Briefly, single-stranded sense DNA with the same sequence as chum salmon VT-I mRNA was prepared by PCR amplification and used as the standard DNA. The 1/20 volume of total RNA from individuals and the serially diluted standard DNA were electrophoresed in a 1% agarose/formaldehyde gel, and transferred to a membrane as described above. Preparation of cDNA probe, hybridization, wash, and exposure were similarly carried out as in the fieldwork. In the 1995 experiment, we adopted the primer extension method instead of the random priming method for the synthesis of the cDNA probe to improve the specificity of hybridization, so that the levels of VT mRNA could be quantitatively analyzed by a dot blot hybridization. The 1/100 volume of total RNA and the serially diluted standard DNA were blotted onto a membrane using a MilliBlot-D (Millipore). The cDNA probe was prepared by the primer extension method using a Megaprime DNA labeling system and [$\alpha$-$^{32}$P]dCTP (Amersham) with a synthetic oligonucleotide primer for VT-I (5'-CTGAAGGCTACTGAGCAC-3') and chum salmon VT-I cDNA as a template. The membranes were hybridized with the labeled probe at 65°C for 20 hr and were washed twice with 0.1× SSPE/0.1% SDS at 60°C for 15 min and exposed to a Fuji imaging plate for 4 hr. Hybridization signals were analyzed by a Bioimaging analyzer (Fuji Photo Film). The levels of VT mRNA were estimated from the standard curve. All samples were analyzed in a single assay within each year to reduce inter-assay variation. For the dot blot analysis, the intra- and inter-assay C.V. were 9.2% and 8.9%, respectively.

**Statistical analyses**

Values are presented as means ± standard errors of the means. Statistical analyses were carried out using one-way ANOVA followed by Tukey's test for multiple comparison (within years or treatments) and Student's *t*-test (between years or treatments). In the 1994 transfer experiment, data of the fish on day 4 of SW-retaining were excluded from statistical analysis, because of the insufficient sample number caused by high mortality in the SW fish.

## RESULTS

### Regional Oceanographic Conditions

In late November from 1992 to 1995, the distribution of the Kurile Current and the Japan Current showed annual variations. In 1992 and 1994, the Japan Current dominated and prevented the south-west branch of Kurile Current from reaching the Sanriku coast (Fig. 1A, C). In contrast, in 1993 and 1995, the Kurile Current dominated and its branch reached near the Sanriku coast (Fig. 1B, D).

### Fieldwork in 1992 and 1993

The changes in GSI, a considerable decrease following gradual increase in the males and an increase followed by a small decrease in the females, are reliable indices of gonadal maturation in salmonids. In the male fish, the GSI decreased from Ocean to Bay in 1992, whereas the GSI decreased from Bay to River in 1993 (Fig. 2A). The GSI in the Bay fish were significantly lower in 1992 than in 1993. In the females, the GSI increased from Ocean to Bay in 1993, whereas the GSI in the Ocean fish were already higher in 1992 than in 1993 (Fig. 2B). Judging from the fact that GSI decreased earlier in 1992 than in 1993 in the males and increased earlier in 1992 than in 1993 in the females, gonadal maturation was almost completed in 1992 but not in 1993 when the fish arrived near their natal river.

In 1992, the plasma $Na^+$ levels were still high in the SW environment (Ocean and Bay) in both sexes, that is, the levels in the Bay fish were 194.7±5.2 mmol/l in the males and 201.6±3.4 mmol/l in the females (Fig. 2C, D). In contrast, in 1993, the plasma $Na^+$ levels were lowered during transition from the Ocean to the Bay in the male fish (Fig. 2C), while the levels had been lowered in the female Ocean fish (Fig. 2D). After the fish entered the river, the plasma $Na^+$ levels were lowered in both years. Between 1992 and 1993, the plasma $Na^+$ levels were significantly different at each sampling point except for the Ocean male fish. Changes in plasma osmolality were similar to those in the plasma $Na^+$ levels in both years (data not shown). Thus, in 1992, both males and females showed concomitantly advanced changes in the GSI values and the high plasma $Na^+$ levels in the SW environment. These facts indicate that SW tolerance was lowered in fully mature fish.

Changes in the VT mRNA levels showed different patterns between 1992 and 1993 in both sexes (Fig. 3A, B). In the males, the VT mRNA levels increased from the Ocean to the River only in 1992 (Fig. 3A). In the females, the VT mRNA levels were lowered in the Bay fish in 1993 (Fig. 3B).

In 1993, plasma DHP levels were significantly elevated during transition from the Ocean to the Bay, and remained high in the River in both sexes (Fig. 3C, D). The magnitudes of elevation in the DHP levels were markedly different between two sexes; the levels in the River fish reached to 19.9±2.4 ng/ml in the males and 215.3±39.6 ng/ml in the females.

### Transfer experiments in 1994 and 1995

The GSI on the initial day of transfer experiments were not significantly different between 1994 and 1995. The GSI did not change significantly within 3 or 4 days of experiments in both years (data not shown). The levels of gonad maturity, however, seemed to be different between 1994 and 1995 at least in the females. In 1994, gonadal maturation seemed to be almost completed, since almost all the fish ovulated on day 4 of both SW-retaining and FW-transfer. In contrast, in 1995, the gonads were still developing, since only two of eight female fish ovulated on day 3 of experiment.

Changes in plasma $Na^+$ levels in the SW-retained fish varied between the two years. In 1994, the plasma $Na^+$ levels in the SW fish were kept high and did not change significantly

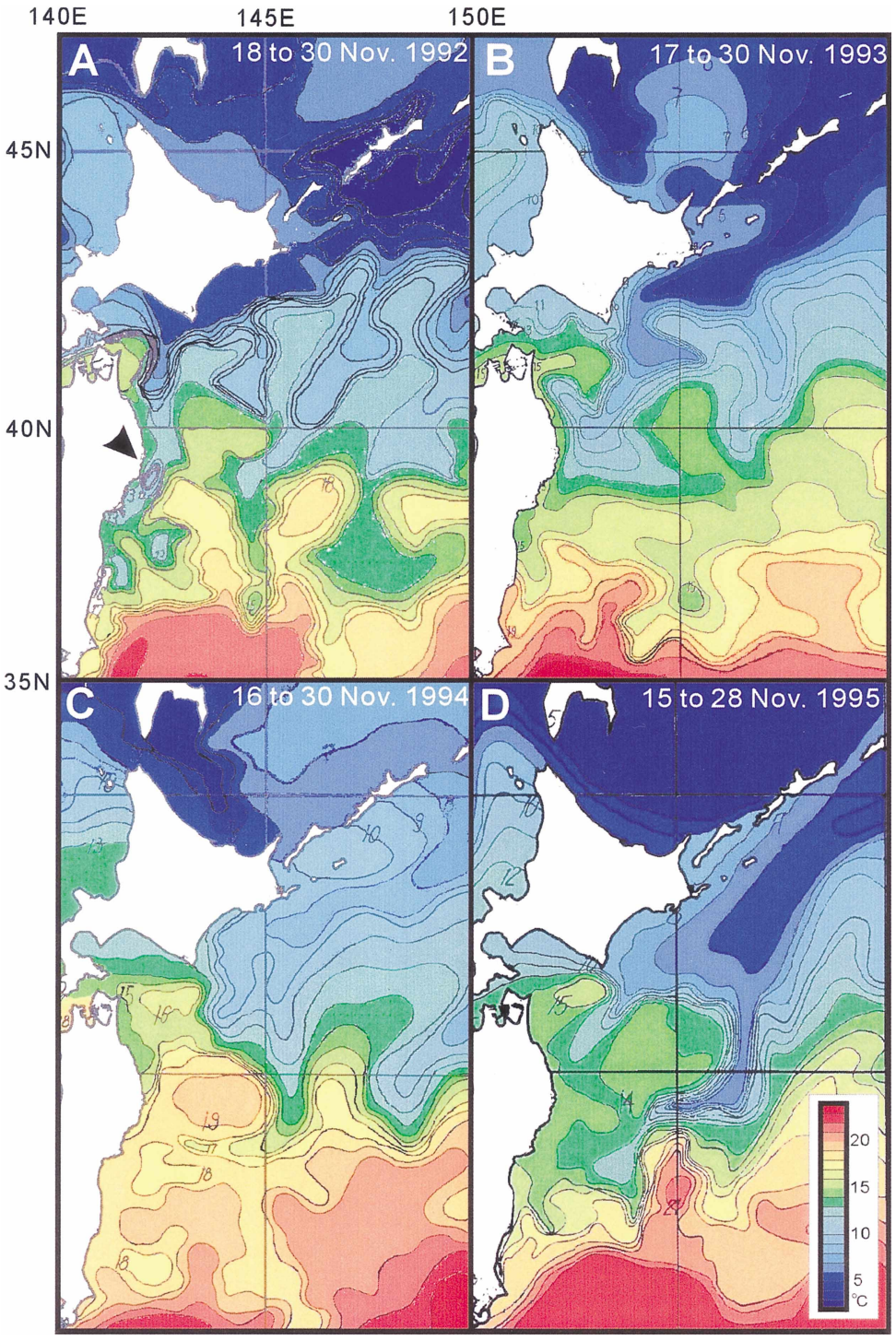


**Fig. 1.** Pseudo color images showing the distribution of mean sea surface temperature in north-west Pacific Ocean near Japan in late November, 1992 (A), 1993 (B), 1994 (C) and 1995 (D). Contour interval is 1°C. The arrowhead indicates the location of the Otsuchi river.

(Fig. 4A, B). The males showed higher mortality; only two of six fish survived on day 4 of SW-retaining. The high plasma $Na^+$ levels and mortality in SW-retained fish indicated a loss of SW tolerance in fully mature fish as was seen in the field-work in 1992. In 1995, plasma $Na^+$ levels in SW fish significantly decreased in the males and reached 170.2±4.4 mmol/l on day 3 (Fig. 4C, D). Also in the females the levels decreased to 172.5±4.6 mmol/l on day 3, although not significant. Transfer to FW decreased the plasma $Na^+$ levels within one day in both years, and the levels in FW fish were lower in 1995 than in 1994.

Changes in the VT mRNA levels were different among years. In 1994, the VT mRNA levels markedly increased throughout 2 days in the SW-retained males (Fig. 5A), whereas

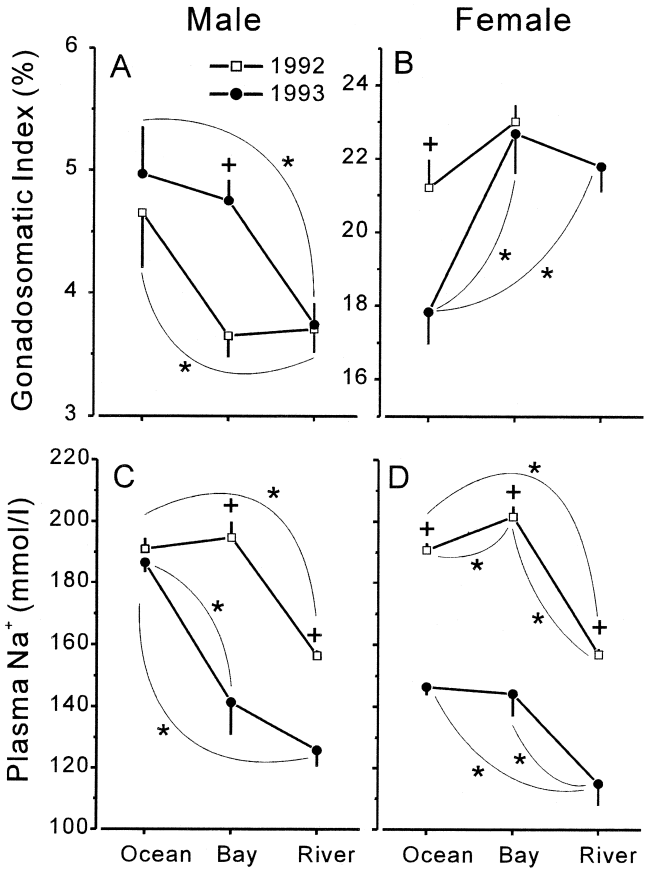


**Fig. 2.** Changes in gonadosomatic index (A, male; B, female) and plasma $Na^+$ levels (C, male; D, female) during the final stages of spawning migration in the fieldwork in 1992 (□) and 1993 (●). Values are means±SEM. n=9–15 (1992) and 7–11 (1993). *$P$<0.05 significantly different within years (one-way ANOVA followed by Tukey's test for multiple comparison). +$P$<0.05 significantly different between years (Student's $t$-test).

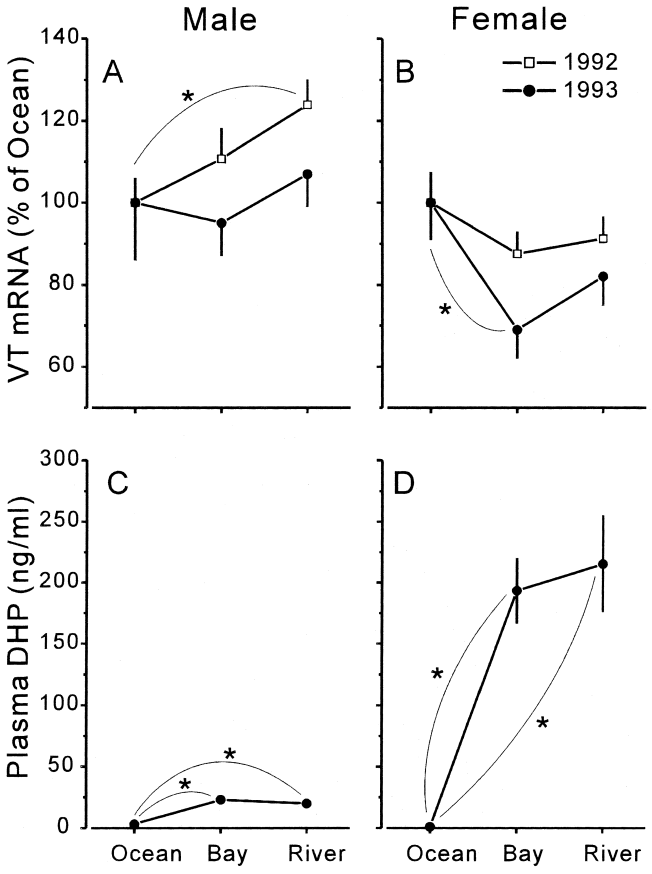


**Fig. 3.** Changes in VT mRNA levels and plasma DHP levels (only in 1993) during the final stages of spawning migration in the fieldwork in 1992 (□) and 1993 (●). Values are means±SEM. n=6–8 (1992) and 7–11 (1993). *$P$<0.05 significantly different within years (one-way ANOVA followed by Tukey's test for multiple comparison).

the levels did not change significantly in the SW-retained females (Fig. 5B). The VT mRNA levels decreased after FW-transfer in both sexes. In 1995, neither SW-retaining nor FW-transfer changed VT mRNA levels in both sexes (Fig. 5C, D).

Like the changes in the VT mRNA levels, plasma DHP levels showed notable changes only in 1994. In 1994, FW-transfer elevated the plasma DHP levels within 2 days in both sexes (Fig. 6A, B). The levels reached 25.6±3.5 ng/ml in the males and 200.3±56.4 ng/ml in the females on day 2 of FW. In 1995, the plasma DHP levels remained unchanged in both FW-transferred and SW-retained fish (Fig. 6 C, D).

## DISCUSSION

The present experiments showed that annually varied regional oceanographic conditions changed the maturity and salinity tolerance in homing salmon, which in turn altered the osmotically-stimulated expression pattern of VT gene. In 1992 and 1994, the influence of Japan Current dominated, whereas in 1993 and 1995, the branch of Kurile Current reached near the Sanriku coast. Homing fish almost completed gonadal maturation and faced to a loss of salinity tolerance in 1992 and 1994. In the 1994 transfer experiment, VT mRNA levels markedly increased in the SW-retained males, whereas the levels were decreased by FW-transfer in both sexes. Homing fish were not fully mature in 1993 and 1995. VT mRNA levels did not show significant changes in both SW-retained and FW-transferred fish in the 1995 transfer experiment.

The coastal oceanographic conditions may have a considerable effect on the accessibility of homing fish to their natal river. The warm current dominated in 1992 and 1994, whereas the branch of cold current reached the Sanriku coast in 1993 and 1995. Correspondingly, the return rates of hatchery-reared chum salmon in the Sanriku coast showed annual variation, that is, from 1992 to 1995 the rates were 2.8%, 3.4%, 2.8%, and 3.2%, respectively (from the statistics on salmon resources in Iwate prefecture). It is generally considered that the branch of Kurile Current, which eventually reaches near the Sanriku coast, provides the homing route for chum salmon returning to the Sanriku coast. Thus, when the Japan Current still dominated on the Sanriku coast, the homing fish were prevented from reaching their natal river, and, as a result, almost completed final maturation when they arrived the coastal area as was seen in 1992 and 1994.

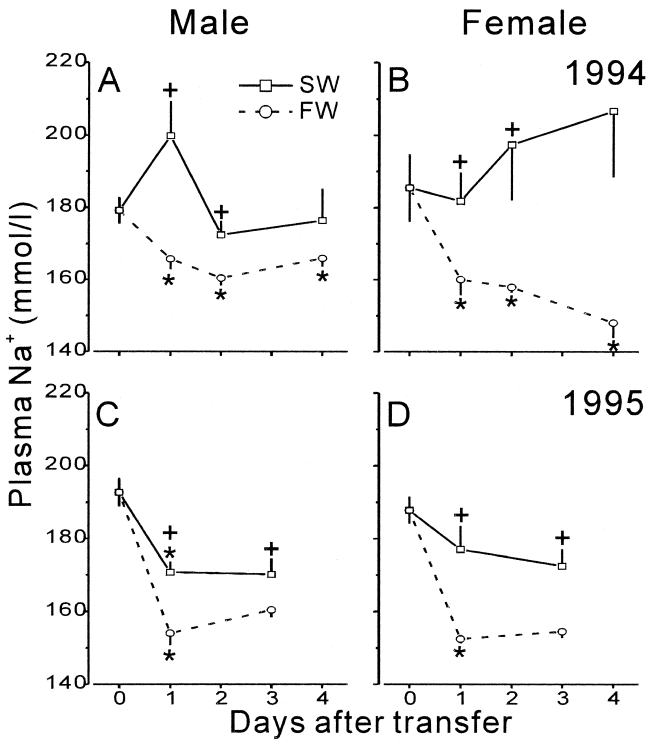


**Fig. 4.** Changes in plasma $Na^+$ levels after transfer to SW (□) or FW (○) environment in 1994 (A, male; B, female) and 1995 (C, male; D, female). Values are means±SEM. n=5–7 (1994) and 5–9 (1995), except for the SW-retained fish on day 4 in 1994 (male, n=2; female, n=4), which were excluded from the statistical analyses. *$P$<0.05 significantly different compared to day 0 (one-way ANOVA followed by Tukey's test for multiple comparison). +$P$<0.05 significantly different between treatments (Student's $t$-test).

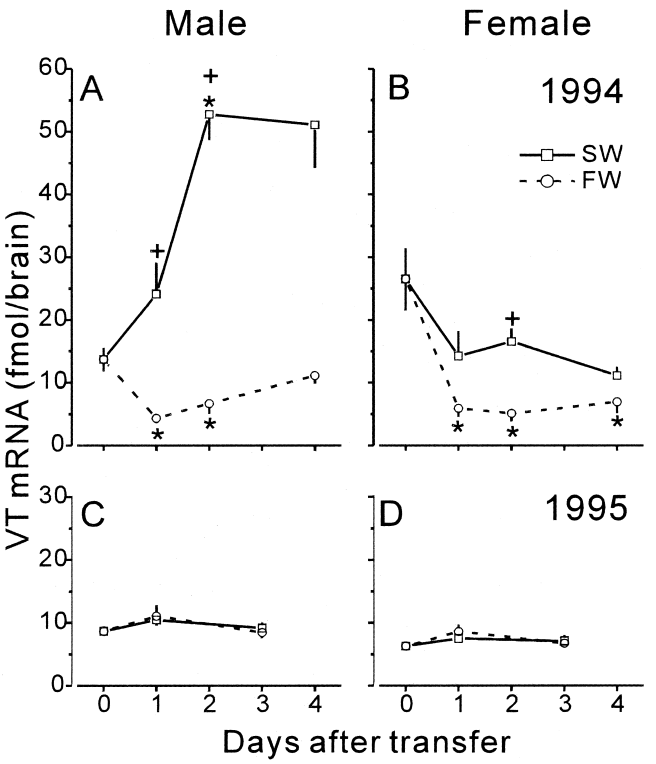


**Fig. 5.** Changes in VT mRNA levels after transfer to SW (□) or FW (○) environment in 1994 (A, male; B, female) and 1995 (C, male; D, female). Values are means±SEM. n=5–7 (1994) and 5–9 (1995), except for the SW-retained fish on day 4 in 1994 (male, n=2; female, n=4), which were excluded from the statistical analyses. *$P$<0.05 significantly different compared to day 0 (one-way ANOVA followed by Tukey's test for multiple comparison). +$P$<0.05 significantly different between treatments (Student's $t$-test).

We adopted different procedures to determine the VT mRNA levels in the transfer experiments in 1994 and 1995. Nonetheless, we consider that the comparison between two years is adequate. The pattern of changes might not be affected by the assay procedure itself, since the levels of VT mRNA were determined in the single assay within each year. Further, the single-stranded sense DNA used as the standards for quantitative analysis was prepared by the same procedure in both years. The coincidence of hybridization signals between the single-stranded sense DNA and synthesized VT sense mRNA was tested previously (Hiraoka *et al.*, 1997). Thus, the estimated values are enough reliable to compare the pattern of changes between two years.

In the 1995 transfer experiment when the fish were not fully mature, the VT mRNA levels did not change after FW-transfer and SW-retaining, in contrast to the results of 1994 experiment in which the fish were fully mature. Interestingly, the plasma $Na^+$ levels lowered toward the FW level in the 1995 SW-retained fish. In the fieldwork, the plasma $Na^+$ levels also decreased in the SW environment in 1993 when the fish were not fully mature. Possibly, in 1993 and 1995, homing fish anticipated the forthcoming FW environment and actively adjusted plasma $Na^+$ levels toward the FW levels just before the fish enter the river. As a result, hypoosmotically-induced decrease in VT gene expression was not observed in these fish. Since homing salmon must undergo a range of changes in salinity and temperature during the homing activity, such anticipatory decreases in plasma $Na^+$ levels, if exist, may help attenuating osmotic shock and maintaining the internal milieu favorable for homing olfaction and gonadal maturation.

In the 1994 experiment when fish were fully mature, the VT mRNA levels increased in the SW-retained males. It is noted that only males showed higher mortality. In brook trout, survival of mature males in SW was significantly poorer than that of females or immature males, suggesting that the effect of lowered salinity tolerance was sexually different (McCormick and Naiman, 1985). Lowered salinity tolerance and consequent mortal stress are possible factors responsible for the remarkable increase in the VT mRNA levels in fully mature, SW-retained males. In the trout pituitary, VT with corticotropin-releasing hormone possesses a synergizing effect on adrenocorticotropin secretion (Baker *et al.*, 1996). Thus, the expression of VT gene can be enhanced in response to the salinity stress in fully mature males.

Our previous studies have shown sexually different expression of VT gene in pre-spawning chum salmon homing to the Ishikari river in Hokkaido. Hypothalamic expression of VT gene was decreased in the females at the final stages of matu-

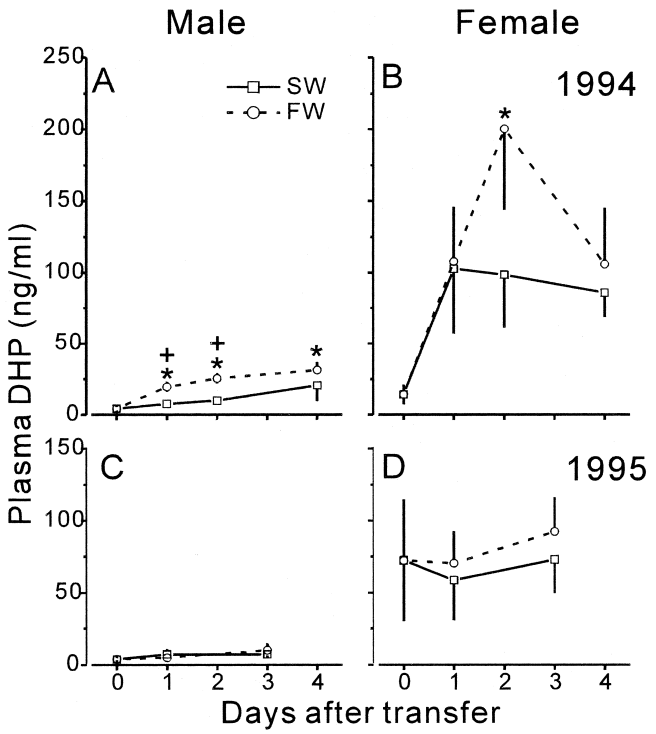


**Fig. 6.** Changes in plasma DHP levels after transfer to SW (□) or FW (○) environment in 1994 (A, male; B, female) and 1995 (C, male; D, female). Values are means±SEM. n=5–7 (1994) and 5–9 (1995), except for the SW-retained fish on day 4 in 1994 (male, n=2; female, n=4), which were excluded from the statistical analyses. *$P$<0.05 significantly different compared to day 0 (one-way ANOVA followed by Tukey's test for multiple comparison). +$P$<0.05 significantly different between treatments (Student's $t$-test).

ration, as shown by Northern blot analysis (Hiraoka *et al.*, 1997) and by *in situ* hybridization analysis (Ota *et al.*, 1996). Furthermore, Ota (1999) showed that the female-specific decrease in the VT mRNA levels occurred when the fish reached spawning beds near the upstream hatchery, whereas the levels did not change soon after the fish entered the Ishikari river. In contrast, in the present fieldwork, such a decrease occurred before the fish entered the Otsuchi river, in which the distance from the river mouth to the spawning ground is much shorter than that in the Ishikari river. Thus, regardless of environmental salinity, the female-specific decrease in VT gene expression occurred just before fish reached the spawning ground, suggesting involvement of VT in reproduction.

Gonadal steroids are involved in regulation of sexually different expression of neurohypophysial hormone genes (see Adan and Burbach, 1992). In masu salmon, seasonal increases in plasma estradiol and testosterone levels were coincident with the increase in the VT mRNA hybridization signals and VT immunoreactivity, suggesting that sex steroids enhanced VT synthesis in pre-mature fish (Ota *et al.*, 1999a, b). Effects of gonadal steroids on synthesis and secretion of neurohypophysial hormones also have been reported in mammals. Testosterone inhibited and estradiol stimulated a vasopressin release in gonadectomised rats (Skowsky *et al.*, 1979). Testosterone increased the vasopressin mRNA levels in castrated and osmotically challenged rats (Crowley and Amico, 1993). In pre-spawning chum salmon, however, the plasma levels of estradiol and testosterone already decreased during the final phases of sexual maturation (Ueda *et al.*, 1984; Ota, 1999). Therefore, they cannot be involved in sexually different VT gene expression at the final phases of spawning migration.

The plasma DHP levels were elevated during transition from the Ocean to the Bay in the fieldwork in 1993 and after FW-transfer in the transfer experiment in 1994. The magnitudes of elevations were markedly different between two sexes. The female-specific elevation in plasma DHP levels at the final stages of gonadal maturation in several salmonid species have been reported (Wright and Hunt, 1982; Ueda *et al.*, 1984). In the present study, the elevation of plasma DHP was coincident with the decrease in the VT mRNA levels. Such coincidence was also observed in pre-spawning chum salmon homing to the Ishikari river (Ota, 1999). Thus the elevation of plasma DHP may be involved in suppression of VT gene expression at the final stages of maturation. These lines of evidence, however, only showed a parallel relationship between plasma DHP levels and VT gene expression, at present. As is discussed above, other steroid hormones, such as sex steroids and cortisol, can be involved in the regulation of VT gene expression (Ota *et al.*, 1999a, b). To clarify the modulatory effects of steroid hormones on VT gene expression, *in vitro* and *in vivo* examinations of causal relationship remain to be conducted.

In conclusion, the regional oceanographic conditions affected the maturity and salinity tolerance in homing salmon, which in turn altered the osmotically-induced expression pattern of VT gene in pre-spawning chum salmon.

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